IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

Chee, M.

Application No.: 09/381,480

Filed: September 16, 1999

For: ITERATIVE RESEQUENCING

Examiner: Chakrabarti, A.

Art Unit: 1634

APPEAL BRIEF

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PATENT
018547-030530US

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On: October 23, 2003

TOWNSEND and TOWNSEND and CREW LLP

By: Kristi Cope

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APPEAL BRIEF

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

Further to the Notice of Appeal filed June 19, 2003, this brief is submitted in appeal of the final rejection mailed March 25, 2003 in the above-captioned case.

I. REAL PARTY IN INTEREST

Affymetrix, Inc.

II. RELATED APPEALS AND INTERFERENCES

None

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III. STATUS OF CLAIMS

Claims 1-15 are pending. All rejected claims are appealed. The claims are listed in Appendix A.

IV. STATUS OF AMENDMENTS

A final office action was mailed March 25, 3003. All previously filed amendments have been entered. No amendment has been filed in response to the final rejection.

V. SUMMARY OF THE PRESENTLY CLAIMED INVENTION

The invention as defined by claim 1 is directed to methods of analyzing a target nucleic acid that is a variant of known reference sequence by iterative steps of array design, hybridization and estimation of the target sequence (see, e.g., specification at p. 7, lines 6 to 26). The method begins with designing an array of probes to comprise a set of probes complementary to a known reference sequence. The claims specifically exclude arrays in which every possible probe sequence of a given length is present (see steps (b) and (e) of claim 1 and specification at p. 10, lines 17-18). The reference sequence serves as a first estimate of the target sequence. The array is then hybridized to the target nucleic acid and the relative hybridization of the probes to the target nucleic acid is determined. The sequence of the target nucleic acid is estimated from the relative hybridization of the probes. A further array of probes is provided comprising a probes set of probes complementary to the estimated sequence of the target nucleic acid. The further array of probes is hybridized with the target nucleic acid and the relative hybridization of the probes is determined. The sequence of the target nucleic acid is reestimated from the relative hybridization of the probes (specification at p. 7, lines 19-23).

Claim 2 specifies further iterations of providing an array comprising probes complementary to an estimate of the target sequence and using the array to reestimate the target sequence until the reestimated sequence of the target nucleic acid is constant between successive cycles (see specification at p. 7, lines 23-26).

Claim 3 specifies that the target sequence is a species variant of a reference sequence. Claim 4 specifies that the reference sequence is from a human and the target nucleic acid is from a primate (see specification at p. 8, lines 12-19).

Claim 12 specifies a preferred configuration for the array of probes used in estimation of the target sequence (see specification at pp. 10-11). The array comprises four probes sets. The first probe set comprises a plurality of probes, each probe comprising a segment of at least six nucleotides exactly complementary to a subsequence of the reference sequence, the segment including at least one interrogation position complementary to a corresponding nucleotide in the reference sequence. The second, third and fourth probe sets each comprise a corresponding probe for each probe in the first probe set, the probes in the second, third an fourth probe sets being identical to a sequence comprising the corresponding probe from first probe set or a subsequence of at least six nucleotides thereof that includes that the at least one interrogation position, except that the at least one interrogation position is occupied by a different nucleotide in each of the four corresponding probes from the four probe sets.

Claim 13, which depends from claim 12, specifies a preferred method of analyzing the type of array recited in claim 12 (specification at p. 11, lines 5-31). In this method, one compares the relative specific binding of four corresponding probes from the first, second, third and fourth probe sets. One then assigns a nucleotide in the sequence of the target nucleic acid as the complement of the interrogation position of the probe having the greatest specific binding. One then repeats these steps until each nucleotide of interest in the sequence of the target nucleic acid has been estimated.

Claim 15, an independent claim, is directed to an iterative method of analyzing a target sequence (see specification at p. 7, lines 6-26). The method includes steps of designing an array of probes to be complementary to an estimated sequence of the target nucleic acid, wherein the array does not contain every possible probe sequence of a given length. The array of probes is then hybridized to a target nucleic acid. One then reestimates the sequence of the target nucleic acid from the hybridization pattern of the array. A further array of probes is then designed to be complementary to the reestimated sequence, and the hybridization and reestimating steps are repeated at least once.

VI. ISSUES

1. Whether claims 1, 2, 5-6 and 15 would have been obvious under 35 USC 103(a) over Skiena, US 5,683,881 (Skiena) in view of Futreal, US 6,045,997 (Futreal) in further view of Cantor, US 5,795,714 (Cantor).

2. Whether claims 7-14 would have been obvious under 35 USC 103(a) over Skiena in view of Futreal in further view of Cantor, in further view of Cronin, US 6,027,880 (Cronin).

3. Whether claims 3 and 4 would have been obvious under 35 USC 103(a) over Skiena in view of Futreal, in further view of Cantor in further view of Horwitz, *J. Virol.* 66, 2170-2179 (1992) (Horwitz).

VII. GROUPING OF THE CLAIMS

The rejected claims do not stand or fall together. As can be seen from the statement of issues, different rejections have been applied to different claims. Also, certain dependent claims are patentable on additional grounds, as discussed in more detail below.

VIII. ARGUMENT

Issue 1: Claims 1, 2, 5-6 and 15 Would Not Have Been Obvious under 35 USC 103(a) over Skiena in view of Futreal in Further View of Cantor

1. The Examiner's Rationale

The Examiner's rationale is most recently stated in the final office action of March 25, 2003. The Examiner takes the view that Skiena teaches each element of claim 1 except for "the array of probes wherein the sequence of the target nucleic acid is a variant of the reference sequence" (final office action at p. 3) and a probe array which does not contain every possible probe sequence of a given length (final office action at p. 4). The Examiner cites Futreal as teaching an array of probes wherein the sequence of the target nucleic acid is a variant of the reference sequence (citing to the Abstract, Figures 1, 2 and 4 and col. 10, lines 23, 27). The Examiner takes the view that it would have been obvious to combine Futreal with Skiena for the advantage disclosed by Futreal that probes are useful in screening assays (final office action at p. 4). The Examiner takes the view that it would have been obvious to combine Cantor's teaching of using incomplete arrays for the benefit of analyzing very rare target nucleic acids in which

nonspecific hybridization is not expected to be a problem or sequencing smaller nucleic acids where the chance of requiring certain combinations of nucleotides is so low as to be practically nonexistent (final office action at p. 5).

2. The Cited Art

Skiena discusses a method of sequencing by hybridization that is intended to be general to any type of target sequence. Initially, the target sequence is hybridized to a universal sequencing array containing all probes of a given length (col. 6, lines 41-42). Subsets of positively and negatively hybridizing probes are then determined (col. 6, lines 45-48). A second array is then designed based on combinations of probes from the positively hybridizing subset (col. 6, lines 49-61). The hybridization is then repeated and subsets of positively and negatively hybridizing probes again determined (col. 6, line 61 to col. 7, line 5). The process is repeated until the cumulative hybridization data reveals the identity of the target sequence (col. 7, lines 9-15).

Futreal is a voluminous patent directed to the cloning of the BRCA2 cancer susceptibility gene. As in most patents dealing with new genes, Futreal provides sequences of the new gene and the protein encoded by it (e.g., Figs. 1, 2 and 4) and mentions that probes can be used to analyze it (col. 9, line 5-21 and col. 10, lines 23-37).

Cantor discusses sequencing by hybridization using arrays of probes having double-stranded and single-stranded regions (col. 7, lines 11-22). Cantor discloses that such arrays can be complete arrays of probes. Cantor also mentions that incomplete arrays can be used for screening procedures of very rare target nucleic acids where nonspecific hybridization is not expected to be a problem or when detecting or sequencing smaller nucleic acids when the chance of requiring certain combination of nucleotides is so low as to be practically nonexistent (col. 13, lines 23-32). Cantor does not say which probes should be omitted from complete arrays in these circumstances.

3. The Burden of Proof

In proceedings before the Patent and Trademark Office, the examiner bears the burden of establishing a prima facie case of obviousness based upon the prior art (*In re Piasecki,* 1471-72,

223 USPQ 785, 787-88 (Fed. Cir. 1984)). To reject claims in an application under section 103, an examiner must show an unrebutted prima facie case of obviousness. In the absence of a prima facie case of obviousness, an appellant who complies with the other statutory requirements is entitled to a patent. *In re Rouffet,* 47 USPQ2d 1453, 55 (Fed. Cir. 1998). If the evidence is in "equipoise," an inventor is "entitled to a patent." *In re Oetiker,* 24 USPQ2d 1443, 1447 (Fed. Cir. 1992) (Plager, J., concurring).

4. Combination of the References Does Not Discloses All Elements of the Claimed Invention

The prior art references when combined must teach or suggest all of the claim limitations. *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991). Here, there are several claim limitations that are not disclosed by the references either individually or in combination.

None of the references discloses step (c) of claim 1 referring to estimating the sequence of a target nucleic acid. The Examiner relies entirely on Skiena for this step, taking the view that such is disclosed by steps (c) and (d) of claim 1 of Skiena. However, neither of these steps recites "estimating" a sequence. Indeed the Board will not find the word "estimating" in the entire Skiena patent. Rather claim 1(c) of Skiena requires identifying a set of hybridizing oligonucleotides, and step (d) recites selecting a second set of oligonucleotides based on the hybridization of the first set. Neither step (c) or (d) refers in any way to the reconstruction of an estimated sequence from the set of positively hybridizing oligonucleotides. To say that a set of positively hybridizing oligonucleotides itself constitutes a sequence without any attempt being made to orient the oligonucleotides with respect to each other would be akin to saying that a restriction mapping of a target reveals its sequence. Such would be abhorrent to usage in the art whereby a restriction map or set of hybridizing oligonucleotides may be regarded as being a fingerprint but is not a sequence.

None of the references discloses step (h) of claim 1, referring to reestimating the sequence of a target nucleic acid. The Examiner again relies entirely on Skiena for such disclosure, taking the view that such is disclosed by claims 1h and 2 of Skiena. However, these claims refer to "determining" the sequence of a target not reestimating it. In Skiena's initial iterations of his method, he does not disclose estimating a target sequence but rather identifies a

subset of hybridizing probes. In the final step of Skiena's method he does not estimate a target sequence, but rather determines the sequence uniquely. In Skiena's view at least, the determined sequence is correct and not an estimated, much less a reestimated sequence (col. 7, lines 12-15).

None of the cited references discloses the step recited in present claim 2 referring to repeating steps (e) and (h) of claim 1, as necessary until the reestimated sequence is constant between successive cycles of the method. The Examiner relies entirely on Skiena for such disclosure, taking the view that such is disclosed by claim 2 of Skiena. However, claim 2 refers to determining a target sequence based on sets of hybridizing probe sets from iterations of his method. In Skiena's method, all cycles but the last determine different subsets of hybridizing probes. The last cycle determines a unique target sequence. There is no mention of estimating target sequences in any cycle, much less of performing the method until an estimated target sequence remains constant.

The Examiner's position may in part be based on the view that simply determining a set of hybridizing oligonucleotides itself constitutes "estimating the sequence of a target," under a broad interpretation of the claims which the Examiner feels entitled to make during prosecution. In response, appellant submits that the Examiner is not merely interpreting the claims broadly, but effectively reading out explicitly recited claim steps. The present claims recite separate steps of "determining the relative hybridization of the probes to the target nucleic acid," and "estimating the sequence of the target nucleic acid from the relative hybridization of the probes." Thus, to view "determining the relative hybridization of probes" as being equivalent to estimating a sequence effectively reads out the step of "estimating the sequence" from the claim.

In attempting to rebut appellant's position, the Examiner states that Skiena "inherently teaches both steps of hybridization and estimation the sequence of a target nucleic acid (citing to col. 9, lines 33-49 and col. 4, lines 19-21) (final office action at p. 10). However, "[i]nherency ... *may not be established by probabilities or possibilities.*" *Mehl/Biophile v. Milgraum,* 52 USPQ2d 1303, 1305 (Fed. Cir. 1999) (emphasis supplied). "The mere fact that a certain thing *may* result from a given set of circumstances *is not sufficient* to establish inherency." *In re Rijckaert,* 28 USPQ2d 1955 (Fed. Cir. 1993) (emphasis supplied). Here, the Examiner's proposal of inherent disclosure of estimating a sequence relies on unsupported assumptions. Thus, when Skiena at col. 4, lines 19-21 refers to resolving "ambiguities" the Examiner is

apparently supposing that Skiena has compiled a sequence from his hybridizing oligonucleotides, and is referring to ambiguities in that sequence. However, there is no basis for such an assumption, particularly, when in the example discussed at col. 6, lines 40 to col. 7, line 9, Skiena does not compile a sequence from hybridizing oligonucleotides until all of the hybridizations have completed. Instead, Skiena's "ambiguities" probably refer to ambiguities in the hybridization data due to the same oligonucleotide being complementary to multiple segments of a target sequence (see col. 4, lines 19-20). Col. 9, lines 33-49 of Skiena merely summarizes Table 2 and the number of iterations of Skiena's method needed to determine a sequence for various targets. This is every reason to suppose that these iterations refer to the same process exemplified at col. 6, lines 40 to col. 7, line 9. As noted in this process, Skiena does not determine a sequence at any iteration except the last.

For these reasons, the Examiner has not established under principles of inherency that Skiena necessarily estimates or reestimates a sequence. Insofar as there is doubt, such doubt should inure to the benefit of appellant given that the burden of proof rests with the PTO. Accordingly, Appellant maintains that Skiena does not teach "estimating," or "reestimating" a target sequence, much less "reestimating" the sequence until it remains constant between successive cycles.

Independent claim 15 is distinguished for at least the same reasons as claims 1. In addition, claim 15 requires iterative steps of designing an array of probes to be complementary to an estimated (or reestimated in subsequent iterations) sequence of a target nucleic acid. Because none of the cited references discloses estimating or reestimating the sequence of a target nucleic acid, it follows that none of the references discloses designing an array of probes to be complementary to an estimated or reestimated sequence of a target nucleic acid.

5. No Motivation to Make the Specific Combination of the References that Would Lead to the Claimed Invention

"To establish a prima facie case of obviousness based on a combination of the content of various references, there must be some teaching, suggestion or motivation in the prior art to make the *specific* combination that was made by the applicant." *In re Dance*, 160 F.3d 1339, 48 USPQ2d 1635, 1637 (Fed. Cir. 1998) (emphasis supplied). The motivation must have sufficient

"force" to "impel persons skilled in the art to do what applicant has done." *Ex parte Levengood,* 28 USPQ2d 1300, 1302 (BPAI 1993).

Here, it is respectfully submitted that the Examiner has not established motivation that would have impelled the artisan to combine the references in the specific manner required by the pending claims. The Examiner cites the following motivation to support combination of Futreal with other references

> An ordinary practitioner would have been motivated to combine and substitute the array of probes wherein the sequence of the target nucleic acids is a variant of the reference sequence of Futreal in the method of Skiena in order to achieve the express advantages noted by Futreal of probes which skilled person is readily able to design, label them and devise suitable conditions for hybridization reactions, assisted by textbooks such as Sambrook et al, and ausubel [sic] et al., and which oligonucleotide probes or primers, as well as the full length sequence (and alleles, variants and derivatives) are also useful in screening a test sample containing a nucleic acid for the presence of alleles and variants, especially those that confer susceptibility or predisposition to cancers, the probes hybridizing with a target sequence from a sample obtained from the individual being tested.
>
> Final office action at p. 3.

However, the asserted motivation represents a benefit of analyzing detecting variants of BRCA2 in general. There are many standard methods for analyzing variants including use of allele-specific probes, allele-specific primers, a single-base extension reaction, dideoxy sequencing, conformational testing, and so forth, some of which are discussed in the Futreal patent. This motivation provides no reason that the skilled artisan would depart from any of the standard methods described in Futreal or textbooks such as Sambrook or Ausubel in favor of that discussed by Skiena. The Skiena method would probably have appeared to be unnecessarily complicated both theoretically and practically for performing a simple task for which many routine methods of analysis were available. Accordingly, it is submitted that the asserted motivation would not have impelled the artisan to combine the teaching of Futreal with Skiena.

It is further submitted that the asserted motivation for combining Cantor with Skiena would not have impelled the combination of reference in the specific manner required by the present claims. The Examiner relies on Cantor's statement that "in certain applications, an entire

array of every possible sequence is not necessary and incomplete arrays are acceptable for use. For example, incomplete arrays may be utilized for screening procedures of very rare target nucleic acids where nonspecific hybridization is not expected to be problematic. Further, every member of an array may not be needed when detecting or sequencing smaller nucleic acids where the chance of requiring certain combinations of nucleotides is so low as to be practically none existence" (final office action at p. 4, citing Cantor at col. 13, lines 23-32).

Although these comments from Cantor may indicate that omission of probes might be feasible within the context of his own methods, they do not demonstrate that an artisan would have been impelled to omit probes and thereby design an array complementary to a reference sequence in the context of Skiena's method. In the context of Cantor's method, omission of any probes from a universal array represents a compromise of competing considerations. Omission of probes is potentially advantageous in that those that remain are fewer in number potentially simplifying construction of the array. However, the omission of probes requires *a priori* assumptions as to the sequence of the target to determine which probes can be omitted. Further, because the probes that can be omitted would vary for different target sequences, omission of probes would require providing different arrays for different target sequences.

The balance of these competing considerations is different in Skiena's method. The iterative strategy proposed by Skiena is designed to resolve ambiguities in the hybridization pattern that may result from the initial hybridization. The capacity to resolve ambiguities in the hybridization pattern at a later stage allows a universal array with relatively few probes (e.g., a C8 universal sequencing array) to be used as the first array in the scheme (see col. 4, lines 11-15). Because the initial array is already relatively small in Skiena's method, there would be correspondingly little economy in effort of synthesis from omission of probes. On the other hand, omission of probes would have severe disadvantages. One would have to make *a priori* assumptions as to which probes do not have complementary sequences in a target and can therefore be omitted. One would also have to synthesize a different starting array for each target sequence, rather than using a single universal array as the method Skiena describes. The extra effort in synthesizing a different starting array for each target sequence would itself more than cancel out any economy of effort saved by reduced synthesis from omission of probes. In the circumstances, it is respectfully submitted that a skilled artisan would not have been impelled by

Cantor's comments to modify Skiena's teaching to design an array of probes to contain probes complementary to a reference sequence as claimed.

6. Conclusion

For each of the above independent and sufficient reasons, appellant respectfully submits the rejection should be reversed.

Issue 2: Claims 7-14 Would Not Have Been Obvious under 35 USC 103(a) over Skiena in view of Futreal in further view of Cantor, in further view of Cronin

Claims 1-2 and 5-15 stand rejected as obvious over Skiena in view of Futreal, in further view of Cantor, in further view of Cronin (final office action at pp. 4-5). Skiena, Futreal and Cantor are applied as above. The Examiner acknowledges that that these references do not teach various details of the reference sequences and probe sets specified in claims 7-14. The Examiner takes the view that these details are described by Cronin. The Examiner takes the view that it would have been obvious to combine the teaching of Cronin with the other references in view of Cronin's statement that her method provides strategies for comparing a target sequence and a reference sequence.

The above claims are distinguished over Cronin, Futreal and Skiena for at least the same reasons as discussed above with respect to Skiena and Futreal alone.

In addition, appellant submits that the asserted motivation would not have impelled an artisan to have combined the teachings of Cronin with Skiena. The motivation identified by the Examiner (that Cronin's method allows one to analyze target sequences that are variants of a reference sequence) provides reason to perform Cronin's methods as written but not to modify them. The proposed motivation also does not take into account the fact that Cronin's strategy of probe design could not be incorporated into Skiena's method without changing much of Skiena's own method and forfeiting most of its advantages. For example, if one employed Cronin's strategy of selecting probe sets by complementarity to a known reference sequence, then one would have to discard Skiena's alternative strategy of using a universal sequence array, and with it, the attendant advantages of being able to analyze any target sequence. It is also unclear why one would employ Skiena' strategy of designing secondary arrays for iterative hybridization if

the primary array were that of Cronin rather than a universal array as described by Skiena. As noted above, Skiena's strategy for designing a secondary array is adapted to analyze a target sequence without any prior knowledge as to its identity. This strategy would have seemed unnecessarily complex compared with the methods that Cronin herself proposes. Thus, it is submitted that asserted motivation of analyzing a target sequence that is a variant of a reference sequence is merely a reason to perform Cronin's own methods, and would not have impelled the artisan to make the specific combination represented by claims 7-14.

The asserted motivation is insufficient for claims 13 for an additional reason. This claim specifies a particular method of analyzing an array containing four probe sets in which one compares the relative specific binding of four corresponding probes from the four probe sets, and assigns a nucleotide in the sequence of the target nucleic acid as the complement of the interrogation position of the probe having the greatest specific binding. The process is then repeated until each nucleotide of interest in the target nucleic acid has been estimated. Although this process is described in the Cronin reference, one would not have been motivated to incorporate it into Skiena's method because it would be redundant in the form of analysis proposed by Skiena. Skiena first determines which probes in a first array hybridize to a target sequence. Skiena then synthesizes a secondary array in which the probes combine the sequences of the hybridizing probes from the first array. Skiena then compiles the sequences from the aggregate of hybridizing probes in all of the arrays. There is no role in this analytical scheme for performing any of the steps recited in claim 13. A skilled artisan would not have been motivated to perform the steps of claim 13 in the context of Skiena's method when the steps of claim 13 serve no purpose in Skiena's method.

For these additional reasons, it is submitted that claims 7-14, and particularly claim 13, are distinguished.

Issue 3: Claims 3 and 4 Would Not Have Been Obvious under 35 USC 103(a) over Skiena in view of Futreal, in further view of Cantor, in further view of Horwitz

Skiena, Futreal and Cantor are applied as above (final office action at p. 7). Horwitz is cited as teaching the identification of homologs of HIV sequences in human and primate DNA. The Examiner takes the view that it would be obvious to combine the comparative human

primate study of Horwitz into Skiena's method for the benefit of obtaining a better understanding of HIV evolution.

Claims 3 and 4 are distinguished for at least the same reasons as claim 1 from which they dependent.

In addition, it is respectfully submitted that the asserted motivation would have been insufficient to have impelled the artisan to combine the references in the specific manner required by the pending claims. The motivation asserted by the Examiner (performing a comparative human primate study) is the reason Horwitz performed his own study of evolution. In this study, Horwitz used conventional Sanger sequencing to sequence particular targets within human and primate genomes. Horwitz provides no indication of inadequacies of this approach, or suggestion that one should turn to any other approach to perform sequencings of cognate sequences from different species. In particular, Horwitz provides nothing that would point the artisan to Skiena's method as a means for analyzing a target nucleic acid that represents a species variant of a known reference sequence. In the absence of any motivation impelling the artisan to the specific combination of Horwitz with Skiena required by claims 2 and 3, it is respectfully submitted that these claims are distinguished on additional grounds.

IX. Conclusion

For all of the above reasons, it is respectfully submitted that the rejections be reversed, and the case remanded to the Examiner for allowance.

Respectfully submitted,

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APPENDIX A: PENDING CLAIMS

1. A method of analyzing a target nucleic acid, comprising:

(a) designing an array of probes to comprise a probe set comprising probes complementary to a known reference sequence;

(b) hybridizing the target nucleic acid to the array of probes, wherein the sequence of the target nucleic acid is a variant of the reference sequence and provided the probe array does not contain every possible probe sequence of a given length;

(c) determining the relative hybridization of the probes to the target nucleic acid,

(d) estimating the sequence of the target nucleic acid from the relative hybridization of the probes;

(e) providing a further array of probes comprising a probe set comprising probes complementary to the estimated sequence of the target nucleic acid and provided the further probe array does not contain every possible probe sequence of a given length;

(f) hybridizing the target nucleic acid to the further array of probes;

(g) determining the relative hybridization of the probes to the target nucleic acid;

(h) reestimating the sequence of the target nucleic acid from the relative hybridization of the probes.

2. The method of claim 1, further comprising repeating steps (e)-(h) as necessary until the reestimated sequence of the target nucleic acid is constant between successive cycles.

3. The method of claim 1, wherein the target nucleic acid is a species variant of the reference sequence.

4. The method of claim 1, wherein the reference sequence is from a human and the target nucleic acid is from a primate.

5. The method of claim 1, wherein the target nucleic acid shows 50-99% sequence identity with the reference sequence.

6. The method of claim 1, wherein the target nucleic acid shows 80-95% sequence identity with the reference sequence.

7. The method of claim 1, wherein the reference sequence is at least 1000 nucleotides long, the array comprises a probe set comprising overlapping probes that are perfectly complementary to and span the reference sequence, and the further array comprises probes that are perfectly complementary to and span the estimated sequence.

8. The method of claim 1, wherein an estimated sequence of the target nucleic acid includes a nucleotide whose identity is ambiguous and the probe set showing perfect complementarity to the estimated sequence includes a probe having a pooled nucleotide aligned with the position of ambiguity in the target sequence.

9. The method of claim 1, wherein the reference sequence is at least 10 kb.

10. The method of claim 1, wherein the reference sequence is at least 1000 kb.

11. The method of claim 1, wherein the reference sequence includes at least 90% of the human genome.

12. The method of claim 1, wherein the array of probes comprises:

(1) a first probe set comprising a plurality of probes, each probe comprising a segment of at least six nucleotides exactly complementary to a subsequence of the reference sequence, the segment including at least one interrogation position complementary to a corresponding nucleotide in the reference sequence,

(2) second, third and fourth probe sets, each comprising a corresponding probe for each probe in the first probe set, the probes in the second, third and fourth probe sets being identical to a sequence comprising the corresponding probe from the first probe set or a subsequence of at least six nucleotides thereof that includes the at least one interrogation

position, except that the at least one interrogation position is occupied by a different nucleotide in each of the four corresponding probes from the four probe sets.

13. The method of claim 12, wherein the sequence of the target nucleic acid is estimated by:

(a) comparing the relative specific binding of four corresponding probes from the first, second, third and fourth probe sets;

(b) assigning a nucleotide in the sequence of the target nucleic acid as the complement of the interrogation position of the probe having the greatest specific binding;

(c) repeating (a) and (b) until each nucleotide of interest in the sequence of the target nucleic acid has been estimated.

14. The method of claim 1, wherein the sequence of the target nucleic acid differs from the reference by at least two positions within a probe length.

15. A method of analyzing a target nucleic acid, comprising:

(a) designing an array of probes to be complementary to an estimated sequence of the target nucleic acid provided the array does not contain every possible probe sequence of a given length,

(b) hybridizing the array of probes to the target nucleic acid;

(c) determining a reestimated sequence of the target nucleic acid from the hybridization pattern of the array to the target nucleic acid sequence to; and

(d) repeating (a)-(c) at least once.

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On: October 23, 2003

TOWNSEND and TOWNSEND and CREW LLP

By: Kristi Cope

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Examiner: Chakrabarti, A.

Art Unit: 1634

APPEAL BRIEF

Commissioner for Patents
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Sir:

Further to the Notice of Appeal filed June 19, 2003, this brief is submitted in appeal of the final rejection mailed March 25, 2003 in the above-captioned case.

I. REAL PARTY IN INTEREST

Affymetrix, Inc.

II. RELATED APPEALS AND INTERFERENCES

None

III. STATUS OF CLAIMS

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A final office action was mailed March 25, 3003. All previously filed amendments have been entered. No amendment has been filed in response to the final rejection.

V. SUMMARY OF THE PRESENTLY CLAIMED INVENTION

The invention as defined by claim 1 is directed to methods of analyzing a target nucleic acid that is a variant of known reference sequence by iterative steps of array design, hybridization and estimation of the target sequence (see, e.g., specification at p. 7, lines 6 to 26). The method begins with designing an array of probes to comprise a set of probes complementary to a known reference sequence. The claims specifically exclude arrays in which every possible probe sequence of a given length is present (see steps (b) and (e) of claim 1 and specification at p. 10, lines 17-18). The reference sequence serves as a first estimate of the target sequence. The array is then hybridized to the target nucleic acid and the relative hybridization of the probes to the target nucleic acid is determined. The sequence of the target nucleic acid is estimated from the relative hybridization of the probes. A further array of probes is provided comprising a probes set of probes complementary to the estimated sequence of the target nucleic acid. The further array of probes is hybridized with the target nucleic acid and the relative hybridization of the probes is determined. The sequence of the target nucleic acid is reestimated from the relative hybridization of the probes (specification at p. 7, lines 19-23).

Claim 2 specifies further iterations of providing an array comprising probes complementary to an estimate of the target sequence and using the array to reestimate the target sequence until the reestimated sequence of the target nucleic acid is constant between successive cycles (see specification at p. 7, lines 23-26).

Claim 3 specifies that the target sequence is a species variant of a reference sequence. Claim 4 specifies that the reference sequence is from a human and the target nucleic acid is from a primate (see specification at p. 8, lines 12-19).

Claim 12 specifies a preferred configuration for the array of probes used in estimation of the target sequence (see specification at pp. 10-11). The array comprises four probes sets. The first probe set comprises a plurality of probes, each probe comprising a segment of at least six nucleotides exactly complementary to a subsequence of the reference sequence, the segment including at least one interrogation position complementary to a corresponding nucleotide in the reference sequence. The second, third and fourth probe sets each comprise a corresponding probe for each probe in the first probe set, the probes in the second, third an fourth probe sets being identical to a sequence comprising the corresponding probe from first probe set or a subsequence of at least six nucleotides thereof that includes that the at least one interrogation position, except that the at least one interrogation position is occupied by a different nucleotide in each of the four corresponding probes from the four probe sets.

Claim 13, which depends from claim 12, specifies a preferred method of analyzing the type of array recited in claim 12 (specification at p. 11, lines 5-31). In this method, one compares the relative specific binding of four corresponding probes from the first, second, third and fourth probe sets. One then assigns a nucleotide in the sequence of the target nucleic acid as the complement of the interrogation position of the probe having the greatest specific binding. One then repeats these steps until each nucleotide of interest in the sequence of the target nucleic acid has been estimated.

Claim 15, an independent claim, is directed to an iterative method of analyzing a target sequence (see specification at p. 7, lines 6-26). The method includes steps of designing an array of probes to be complementary to an estimated sequence of the target nucleic acid, wherein the array does not contain every possible probe sequence of a given length. The array of probes is then hybridized to a target nucleic acid. One then reestimates the sequence of the target nucleic acid from the hybridization pattern of the array. A further array of probes is then designed to be complementary to the reestimated sequence, and the hybridization and reestimating steps are repeated at least once.

VI. ISSUES

1. Whether claims 1, 2, 5-6 and 15 would have been obvious under 35 USC 103(a) over Skiena, US 5,683,881 (Skiena) in view of Futreal, US 6,045,997 (Futreal) in further view of Cantor, US 5,795,714 (Cantor).

2. Whether claims 7-14 would have been obvious under 35 USC 103(a) over Skiena in view of Futreal in further view of Cantor, in further view of Cronin, US 6,027,880 (Cronin).

3. Whether claims 3 and 4 would have been obvious under 35 USC 103(a) over Skiena in view of Futreal, in further view of Cantor in further view of Horwitz, *J. Virol.* 66, 2170-2179 (1992) (Horwitz).

VII. GROUPING OF THE CLAIMS

The rejected claims do not stand or fall together. As can be seen from the statement of issues, different rejections have been applied to different claims. Also, certain dependent claims are patentable on additional grounds, as discussed in more detail below.

VIII. ARGUMENT

Issue 1: Claims 1, 2, 5-6 and 15 Would Not Have Been Obvious under 35 USC 103(a) over Skiena in view of Futreal in Further View of Cantor

1. The Examiner's Rationale

The Examiner's rationale is most recently stated in the final office action of March 25, 2003. The Examiner takes the view that Skiena teaches each element of claim 1 except for "the array of probes wherein the sequence of the target nucleic acid is a variant of the reference sequence" (final office action at p. 3) and a probe array which does not contain every possible probe sequence of a given length (final office action at p. 4). The Examiner cites Futreal as teaching an array of probes wherein the sequence of the target nucleic acid is a variant of the reference sequence (citing to the Abstract, Figures 1, 2 and 4 and col. 10, lines 23, 27). The Examiner takes the view that it would have been obvious to combine Futreal with Skiena for the advantage disclosed by Futreal that probes are useful in screening assays (final office action at p. 4). The Examiner takes the view that it would have been obvious to combine Cantor's teaching of using incomplete arrays for the benefit of analyzing very rare target nucleic acids in which

nonspecific hybridization is not expected to be a problem or sequencing smaller nucleic acids where the chance of requiring certain combinations of nucleotides is so low as to be practically nonexistent (final office action at p. 5).

2. The Cited Art

Skiena discusses a method of sequencing by hybridization that is intended to be general to any type of target sequence. Initially, the target sequence is hybridized to a universal sequencing array containing all probes of a given length (col. 6, lines 41-42). Subsets of positively and negatively hybridizing probes are then determined (col. 6, lines 45-48). A second array is then designed based on combinations of probes from the positively hybridizing subset (col. 6, lines 49-61). The hybridization is then repeated and subsets of positively and negatively hybridizing probes again determined (col. 6, line 61 to col. 7, line 5). The process is repeated until the cumulative hybridization data reveals the identity of the target sequence (col. 7, lines 9-15).

Futreal is a voluminous patent directed to the cloning of the BRCA2 cancer susceptibility gene. As in most patents dealing with new genes, Futreal provides sequences of the new gene and the protein encoded by it (e.g., Figs. 1, 2 and 4) and mentions that probes can be used to analyze it (col. 9, line 5-21 and col. 10, lines 23-37).

Cantor discusses sequencing by hybridization using arrays of probes having double-stranded and single-stranded regions (col. 7, lines 11-22). Cantor discloses that such arrays can be complete arrays of probes. Cantor also mentions that incomplete arrays can be used for screening procedures of very rare target nucleic acids where nonspecific hybridization is not expected to be a problem or when detecting or sequencing smaller nucleic acids when the chance of requiring certain combination of nucleotides is so low as to be practically nonexistent (col. 13, lines 23-32). Cantor does not say which probes should be omitted from complete arrays in these circumstances.

3. The Burden of Proof

In proceedings before the Patent and Trademark Office, the examiner bears the burden of establishing a prima facie case of obviousness based upon the prior art (*In re Piasecki,* 1471-72,

223 USPQ 785, 787-88 (Fed. Cir. 1984)). To reject claims in an application under section 103, an examiner must show an unrebutted prima facie case of obviousness. In the absence of a prima facie case of obviousness, an appellant who complies with the other statutory requirements is entitled to a patent. *In re Rouffet,* 47 USPQ2d 1453, 55 (Fed. Cir. 1998). If the evidence is in "equipoise," an inventor is "entitled to a patent." *In re Oetiker,* 24 USPQ2d 1443, 1447 (Fed. Cir. 1992) (Plager, J., concurring).

4. Combination of the References Does Not Discloses All Elements of the Claimed Invention

The prior art references when combined must teach or suggest all of the claim limitations. *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991). Here, there are several claim limitations that are not disclosed by the references either individually or in combination.

None of the references discloses step (c) of claim 1 referring to estimating the sequence of a target nucleic acid. The Examiner relies entirely on Skiena for this step, taking the view that such is disclosed by steps (c) and (d) of claim 1 of Skiena. However, neither of these steps recites "estimating" a sequence. Indeed the Board will not find the word "estimating" in the entire Skiena patent. Rather claim 1(c) of Skiena requires identifying a set of hybridizing oligonucleotides, and step (d) recites selecting a second set of oligonucleotides based on the hybridization of the first set. Neither step (c) or (d) refers in any way to the reconstruction of an estimated sequence from the set of positively hybridizing oligonucleotides. To say that a set of positively hybridizing oligonucleotides itself constitutes a sequence without any attempt being made to orient the oligonucleotides with respect to each other would be akin to saying that a restriction mapping of a target reveals its sequence. Such would be abhorrent to usage in the art whereby a restriction map or set of hybridizing oligonucleotides may be regarded as being a fingerprint but is not a sequence.

None of the references discloses step (h) of claim 1, referring to reestimating the sequence of a target nucleic acid. The Examiner again relies entirely on Skiena for such disclosure, taking the view that such is disclosed by claims 1h and 2 of Skiena. However, these claims refer to "determining" the sequence of a target not reestimating it. In Skiena's initial iterations of his method, he does not disclose estimating a target sequence but rather identifies a

subset of hybridizing probes. In the final step of Skiena's method he does not estimate a target sequence, but rather determines the sequence uniquely. In Skiena's view at least, the determined sequence is correct and not an estimated, much less a reestimated sequence (col. 7, lines 12-15).

None of the cited references discloses the step recited in present claim 2 referring to repeating steps (e) and (h) of claim 1, as necessary until the reestimated sequence is constant between successive cycles of the method. The Examiner relies entirely on Skiena for such disclosure, taking the view that such is disclosed by claim 2 of Skiena. However, claim 2 refers to determining a target sequence based on sets of hybridizing probe sets from iterations of his method. In Skiena's method, all cycles but the last determine different subsets of hybridizing probes. The last cycle determines a unique target sequence. There is no mention of estimating target sequences in any cycle, much less of performing the method until an estimated target sequence remains constant.

The Examiner's position may in part be based on the view that simply determining a set of hybridizing oligonucleotides itself constitutes "estimating the sequence of a target," under a broad interpretation of the claims which the Examiner feels entitled to make during prosecution. In response, appellant submits that the Examiner is not merely interpreting the claims broadly, but effectively reading out explicitly recited claim steps. The present claims recite separate steps of "determining the relative hybridization of the probes to the target nucleic acid," and "estimating the sequence of the target nucleic acid from the relative hybridization of the probes." Thus, to view "determining the relative hybridization of probes" as being equivalent to estimating a sequence effectively reads out the step of "estimating the sequence" from the claim.

In attempting to rebut appellant's position, the Examiner states that Skiena "inherently teaches both steps of hybridization and estimation the sequence of a target nucleic acid (citing to col. 9, lines 33-49 and col. 4, lines 19-21) (final office action at p. 10). However, "[i]nherency *... may not be established by probabilities or possibilities.*" *Mehl/Biophile v. Milgraum,* 52 USPQ2d 1303, 1305 (Fed. Cir. 1999) (emphasis supplied). "The mere fact that a certain thing *may* result from a given set of circumstances *is not sufficient* to establish inherency." *In re Rijckaert,* 28 USPQ2d 1955 (Fed. Cir. 1993) (emphasis supplied). Here, the Examiner's proposal of inherent disclosure of estimating a sequence relies on unsupported assumptions. Thus, when Skiena at col. 4, lines 19-21 refers to resolving "ambiguities" the Examiner is

apparently supposing that Skiena has compiled a sequence from his hybridizing oligonucleotides, and is referring to ambiguities in that sequence. However, there is no basis for such an assumption, particularly, when in the example discussed at col. 6, lines 40 to col. 7, line 9, Skiena does not compile a sequence from hybridizing oligonucleotides until all of the hybridizations have completed. Instead, Skiena's "ambiguities" probably refer to ambiguities in the hybridization data due to the same oligonucleotide being complementary to multiple segments of a target sequence (see col. 4, lines 19-20). Col. 9, lines 33-49 of Skiena merely summarizes Table 2 and the number of iterations of Skiena's method needed to determine a sequence for various targets. This is every reason to suppose that these iterations refer to the same process exemplified at col. 6, lines 40 to col. 7, line 9. As noted in this process, Skiena does not determine a sequence at any iteration except the last.

For these reasons, the Examiner has not established under principles of inherency that Skiena necessarily estimates or reestimates a sequence. Insofar as there is doubt, such doubt should inure to the benefit of appellant given that the burden of proof rests with the PTO. Accordingly, Appellant maintains that Skiena does not teach "estimating," or "reestimating" a target sequence, much less "reestimating" the sequence until it remains constant between successive cycles.

Independent claim 15 is distinguished for at least the same reasons as claims 1. In addition, claim 15 requires iterative steps of designing an array of probes to be complementary to an estimated (or reestimated in subsequent iterations) sequence of a target nucleic acid. Because none of the cited references discloses estimating or reestimating the sequence of a target nucleic acid, it follows that none of the references discloses designing an array of probes to be complementary to an estimated or reestimated sequence of a target nucleic acid.

5. No Motivation to Make the Specific Combination of the References that Would Lead to the Claimed Invention

"To establish a prima facie case of obviousness based on a combination of the content of various references, there must be some teaching, suggestion or motivation in the prior art to make the *specific* combination that was made by the applicant." *In re Dance*, 160 F.3d 1339, 48 USPQ2d 1635, 1637 (Fed. Cir. 1998) (emphasis supplied). The motivation must have sufficient

"force" to "impel persons skilled in the art to do what applicant has done." *Ex parte Levengood,* 28 USPQ2d 1300, 1302 (BPAI 1993).

Here, it is respectfully submitted that the Examiner has not established motivation that would have impelled the artisan to combine the references in the specific manner required by the pending claims. The Examiner cites the following motivation to support combination of Futreal with other references

> An ordinary practitioner would have been motivated to combine and substitute the array of probes wherein the sequence of the target nucleic acids is a variant of the reference sequence of Futreal in the method of Skiena in order to achieve the express advantages noted by Futreal of probes which skilled person is readily able to design, label them and devise suitable conditions for hybridization reactions, assisted by textbooks such as Sambrook et al, and ausubel [sic] et al., and which oligonucleotide probes or primers, as well as the full length sequence (and alleles, variants and derivatives) are also useful in screening a test sample containing a nucleic acid for the presence of alleles and variants, especially those that confer susceptibility or predisposition to cancers, the probes hybridizing with a target sequence from a sample obtained from the individual being tested.
>
> Final office action at p. 3.

However, the asserted motivation represents a benefit of analyzing detecting variants of BRCA2 in general. There are many standard methods for analyzing variants including use of allele-specific probes, allele-specific primers, a single-base extension reaction, dideoxy sequencing, conformational testing, and so forth, some of which are discussed in the Futreal patent. This motivation provides no reason that the skilled artisan would depart from any of the standard methods described in Futreal or textbooks such as Sambrook or Ausubel in favor of that discussed by Skiena. The Skiena method would probably have appeared to be unnecessarily complicated both theoretically and practically for performing a simple task for which many routine methods of analysis were available. Accordingly, it is submitted that the asserted motivation would not have impelled the artisan to combine the teaching of Futreal with Skiena.

It is further submitted that the asserted motivation for combining Cantor with Skiena would not have impelled the combination of reference in the specific manner required by the present claims. The Examiner relies on Cantor's statement that "in certain applications, an entire

array of every possible sequence is not necessary and incomplete arrays are acceptable for use. For example, incomplete arrays may be utilized for screening procedures of very rare target nucleic acids where nonspecific hybridization is not expected to be problematic. Further, every member of an array may not be needed when detecting or sequencing smaller nucleic acids where the chance of requiring certain combinations of nucleotides is so low as to be practically none existence" (final office action at p. 4, citing Cantor at col. 13, lines 23-32).

Although these comments from Cantor may indicate that omission of probes might be feasible within the context of his own methods, they do not demonstrate that an artisan would have been impelled to omit probes and thereby design an array complementary to a reference sequence in the context of Skiena's method. In the context of Cantor's method, omission of any probes from a universal array represents a compromise of competing considerations. Omission of probes is potentially advantageous in that those that remain are fewer in number potentially simplifying construction of the array. However, the omission of probes requires *a priori* assumptions as to the sequence of the target to determine which probes can be omitted. Further, because the probes that can be omitted would vary for different target sequences, omission of probes would require providing different arrays for different target sequences.

The balance of these competing considerations is different in Skiena's method. The iterative strategy proposed by Skiena is designed to resolve ambiguities in the hybridization pattern that may result from the initial hybridization. The capacity to resolve ambiguities in the hybridization pattern at a later stage allows a universal array with relatively few probes (e.g., a C8 universal sequencing array) to be used as the first array in the scheme (see col. 4, lines 11-15). Because the initial array is already relatively small in Skiena's method, there would be correspondingly little economy in effort of synthesis from omission of probes. On the other hand, omission of probes would have severe disadvantages. One would have to make *a priori* assumptions as to which probes do not have complementary sequences in a target and can therefore be omitted. One would also have to synthesize a different starting array for each target sequence, rather than using a single universal array as the method Skiena describes. The extra effort in synthesizing a different starting array for each target sequence would itself more than cancel out any economy of effort saved by reduced synthesis from omission of probes. In the circumstances, it is respectfully submitted that a skilled artisan would not have been impelled by

Cantor's comments to modify Skiena's teaching to design an array of probes to contain probes complementary to a reference sequence as claimed.

6. Conclusion

For each of the above independent and sufficient reasons, appellant respectfully submits the rejection should be reversed.

Issue 2: Claims 7-14 Would Not Have Been Obvious under 35 USC 103(a) over Skiena in view of Futreal in further view of Cantor, in further view of Cronin

Claims 1-2 and 5-15 stand rejected as obvious over Skiena in view of Futreal, in further view of Cantor, in further view of Cronin (final office action at pp. 4-5). Skiena, Futreal and Cantor are applied as above. The Examiner acknowledges that that these references do not teach various details of the reference sequences and probe sets specified in claims 7-14. The Examiner takes the view that these details are described by Cronin. The Examiner takes the view that it would have been obvious to combine the teaching of Cronin with the other references in view of Cronin's statement that her method provides strategies for comparing a target sequence and a reference sequence.

The above claims are distinguished over Cronin, Futreal and Skiena for at least the same reasons as discussed above with respect to Skiena and Futreal alone.

In addition, appellant submits that the asserted motivation would not have impelled an artisan to have combined the teachings of Cronin with Skiena. The motivation identified by the Examiner (that Cronin's method allows one to analyze target sequences that are variants of a reference sequence) provides reason to perform Cronin's methods as written but not to modify them. The proposed motivation also does not take into account the fact that Cronin's strategy of probe design could not be incorporated into Skiena's method without changing much of Skiena's own method and forfeiting most of its advantages. For example, if one employed Cronin's strategy of selecting probe sets by complementarity to a known reference sequence, then one would have to discard Skiena's alternative strategy of using a universal sequence array, and with it, the attendant advantages of being able to analyze any target sequence. It is also unclear why one would employ Skiena' strategy of designing secondary arrays for iterative hybridization if

the primary array were that of Cronin rather than a universal array as described by Skiena.. As noted above, Skiena's strategy for designing a secondary array is adapted to analyze a target sequence without any prior knowledge as to its identity. This strategy would have seemed unnecessarily complex compared with the methods that Cronin herself proposes. Thus, it is submitted that asserted motivation of analyzing a target sequence that is a variant of a reference sequence is merely a reason to perform Cronin's own methods, and would not have impelled the artisan to make the specific combination represented by claims 7-14.

The asserted motivation is insufficient for claims 13 for an additional reason. This claim specifies a particular method of analyzing an array containing four probe sets in which one compares the relative specific binding of four corresponding probes from the four probe sets, and assigns a nucleotide in the sequence of the target nucleic acid as the complement of the interrogation position of the probe having the greatest specific binding. The process is then repeated until each nucleotide of interest in the target nucleic acid has been estimated. Although this process is described in the Cronin reference, one would not have been motivated to incorporate it into Skiena's method because it would be redundant in the form of analysis proposed by Skiena. Skiena first determines which probes in a first array hybridize to a target sequence. Skiena then synthesizes a secondary array in which the probes combine the sequences of the hybridizing probes from the first array. Skiena then compiles the sequences from the aggregate of hybridizing probes in all of the arrays. There is no role in this analytical scheme for performing any of the steps recited in claim 13. A skilled artisan would not have been motivated to perform the steps of claim 13 in the context of Skiena's method when the steps of claim 13 serve no purpose in Skiena's method.

For these additional reasons, it is submitted that claims 7-14, and particularly claim 13, are distinguished.

Issue 3: Claims 3 and 4 Would Not Have Been Obvious under 35 USC 103(a) over Skiena in view of Futreal, in further view of Cantor, in further view of Horwitz

Skiena, Futreal and Cantor are applied as above (final office action at p. 7). Horwitz is cited as teaching the identification of homologs of HIV sequences in human and primate DNA. The Examiner takes the view that it would be obvious to combine the comparative human

primate study of Horwitz into Skiena's method for the benefit of obtaining a better understanding of HIV evolution.

Claims 3 and 4 are distinguished for at least the same reasons as claim 1 from which they dependent.

In addition, it is respectfully submitted that the asserted motivation would have been insufficient to have impelled the artisan to combine the references in the specific manner required by the pending claims. The motivation asserted by the Examiner (performing a comparative human primate study) is the reason Horwitz performed his own study of evolution. In this study, Horwitz used conventional Sanger sequencing to sequence particular targets within human and primate genomes. Horwitz provides no indication of inadequacies of this approach, or suggestion that one should turn to any other approach to perform sequencings of cognate sequences from different species. In particular, Horwitz provides nothing that would point the artisan to Skiena's method as a means for analyzing a target nucleic acid that represents a species variant of a known reference sequence. In the absence of any motivation impelling the artisan to the specific combination of Horwitz with Skiena required by claims 2 and 3, it is respectfully submitted that these claims are distinguished on additional grounds.

IX. Conclusion

For all of the above reasons, it is respectfully submitted that the rejections be reversed, and the case remanded to the Examiner for allowance.

Respectfully submitted,

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APPENDIX A: PENDING CLAIMS

1. A method of analyzing a target nucleic acid, comprising:

(a) designing an array of probes to comprise a probe set comprising probes complementary to a known reference sequence;

(b) hybridizing the target nucleic acid to the array of probes, wherein the sequence of the target nucleic acid is a variant of the reference sequence and provided the probe array does not contain every possible probe sequence of a given length;

(c) determining the relative hybridization of the probes to the target nucleic acid,

(d) estimating the sequence of the target nucleic acid from the relative hybridization of the probes;

(e) providing a further array of probes comprising a probe set comprising probes complementary to the estimated sequence of the target nucleic acid and provided the further probe array does not contain every possible probe sequence of a given length;

(f) hybridizing the target nucleic acid to the further array of probes;

(g) determining the relative hybridization of the probes to the target nucleic acid;

(h) reestimating the sequence of the target nucleic acid from the relative hybridization of the probes.

2. The method of claim 1, further comprising repeating steps (e)-(h) as necessary until the reestimated sequence of the target nucleic acid is constant between successive cycles.

3. The method of claim 1, wherein the target nucleic acid is a species variant of the reference sequence.

4. The method of claim 1, wherein the reference sequence is from a human and the target nucleic acid is from a primate.

5. The method of claim 1, wherein the target nucleic acid shows 50-99% sequence identity with the reference sequence.

6. The method of claim 1, wherein the target nucleic acid shows 80-95% sequence identity with the reference sequence.

7. The method of claim 1, wherein the reference sequence is at least 1000 nucleotides long, the array comprises a probe set comprising overlapping probes that are perfectly complementary to and span the reference sequence, and the further array comprises probes that are perfectly complementary to and span the estimated sequence.

8. The method of claim 1, wherein an estimated sequence of the target nucleic acid includes a nucleotide whose identity is ambiguous and the probe set showing perfect complementarity to the estimated sequence includes a probe having a pooled nucleotide aligned with the position of ambiguity in the target sequence.

9. The method of claim 1, wherein the reference sequence is at least 10 kb.

10. The method of claim 1, wherein the reference sequence is at least 1000 kb.

11. The method of claim 1, wherein the reference sequence includes at least 90% of the human genome.

12. The method of claim 1, wherein the array of probes comprises:

(1) a first probe set comprising a plurality of probes, each probe comprising a segment of at least six nucleotides exactly complementary to a subsequence of the reference sequence, the segment including at least one interrogation position complementary to a corresponding nucleotide in the reference sequence,

(2) second, third and fourth probe sets, each comprising a corresponding probe for each probe in the first probe set, the probes in the second, third and fourth probe sets being identical to a sequence comprising the corresponding probe from the first probe set or a subsequence of at least six nucleotides thereof that includes the at least one interrogation

position, except that the at least one interrogation position is occupied by a different nucleotide in each of the four corresponding probes from the four probe sets.

13. The method of claim 12, wherein the sequence of the target nucleic acid is estimated by:

(a) comparing the relative specific binding of four corresponding probes from the first, second, third and fourth probe sets;

(b) assigning a nucleotide in the sequence of the target nucleic acid as the complement of the interrogation position of the probe having the greatest specific binding;

(c) repeating (a) and (b) until each nucleotide of interest in the sequence of the target nucleic acid has been estimated.

14. The method of claim 1, wherein the sequence of the target nucleic acid differs from the reference by at least two positions within a probe length.

15. A method of analyzing a target nucleic acid, comprising:

(a) designing an array of probes to be complementary to an estimated sequence of the target nucleic acid provided the array does not contain every possible probe sequence of a given length,

(b) hybridizing the array of probes to the target nucleic acid;

(c) determining a reestimated sequence of the target nucleic acid from the hybridization pattern of the array to the target nucleic acid sequence to; and

(d) repeating (a)-(c) at least once.